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Stimulation of parathyroid hormone secretion by phorbol esters is associated with a decrease of cytosolic calcium

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Unlike in other endocrine systems calcium inhibits parathyroid hormone (PTH) secretion and this inhibition is paralleled by a rise of cytosolic calcium concentration ($[Ca]_i$). Because of evidence that diglyceride levels and protein kinase C activity are also decreased by high extracellular calcium we have investigated the effects of 12-*O*-tetradecanoylphorbol-13-acetate (TPA), an activator of protein kinase C, on $[Ca]_i$ and PTH secretion using dispersed bovine parathyroid cells. At 1.5 mM medium calcium TPA enhanced PTH secretion and caused reduction of $[Ca]_i$ from 639 ± 36 nM (SE) to 335 ± 21 nM ($P < 0.001$); at 0.5 mM calcium TPA was ineffective. Moreover, TPA suppressed the rise of $[Ca]_i$ evoked by high extracellular calcium. Thus TPA presumably stimulates PTH secretion via activation of protein kinase C, and the lowering of $[Ca]_i$ may be a secondary event related to diglyceride availability.

Parathyroid hormone *Phorbol ester* *Cytosolic calcium*

1. INTRODUCTION

Phorbol esters activate protein kinase C and by these means interact with secretory events in various endocrine cells (for references see [1]). Recently, Brown et al. [2] have demonstrated stimulation of PTH secretion by TPA at high but not low extracellular calcium concentration. The stimulation of PTH secretion was not associated with changes of cAMP production and apparently cytosolic calcium concentration ($[Ca]_i$) remained unchanged. The authors concluded that protein kinase C is directly involved in the mediation of PTH secretory responses.

It is well established that low extracellular calcium concentration enhances PTH secretion [3], and extracellular calcium and $[Ca]_i$ are proportionally related [4]. The question as to whether changes of $[Ca]_i$ also mediate parathyroid secretory responses or represent a secondary event remains to be elucidated. Here we have confirmed stimulation of PTH secretion by phorbol esters which in our hands, contrary to Brown et al. [2], was associated with a fall of $[Ca]_i$. Moreover, raising extracellular calcium in the presence of TPA abolished the rise of $[Ca]_i$. Activation of protein kinase C by TPA probably enhances PTH secretion directly. Thus changes of $[Ca]_i$ do not necessarily mediate PTH secretory responses.

2. EXPERIMENTAL

Dispersed bovine parathyroid cells [5] were separated from contaminating red and other cells by density gradient centrifugation [6].

For loading with quin2 (obtained from T.J. Rink, University of Cambridge, England) 2–3 × 10^6 parathyroid cells/ml were incubated at 37°C for 15 min with 50 μM quin2-AM, and subsequently the suspension was diluted 10-fold and in-

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Abbreviations: DMSO, dimethyl sulfoxide; PDD, phorbol-12,13-didecanoate; PTH, parathyroid hormone; TPA, 12-*O*-tetradecanoylphorbol-13-acetate

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cubated for an additional 45 min. Cells loaded by this procedure typically contained 0.5 mM quin2. For fluorescence measurements $1-2 \times 10^6$ cells/ml were washed and transferred to a medium containing 0.5 or 1.5 mM $CaCl_2$, and 137 mM NaCl, 5.6 mM KCl, 0.8 mM Na_2HPO_4, 1 mM $MgSO_4$, 5.6 mM glucose, 10 μM diethylenetriaminepentaacetic acid, and 20 mM Hepes, pH 7.45. Fluorescence was measured at 37°C by spectrofluorimetry (Perkin-Elmer type 650-10S) with respective excitation and emission wavelengths of 340 and 490 nm and slit widths of 4 and 10 nm. Cell suspensions were stirred manually every 40–60 s. The quin2 signal was calibrated by addition of 50 μM digitonin for F_{max} and 1 mM Mn^{2+} for autofluorescence (A). $[Ca]_i$ was calculated from the equation: $[Ca]_i = 115\ nM \times (F - F_{min}/F_{max} - F)$, $(F_{min} = A + 1/6/F_{max} - A)$ [7]. The phorbol esters used, TPA, 4α- and 4β-phorbol-12,13-didecanoate (PDD) (Sigma, St. Louis, MO) did not affect quin2 fluorescence. Stock solutions of the phorbol esters were stored in dry DMSO at −20°C and diluted to working concentrations with DMSO. DMSO concentration in the cell suspensions did not exceed 1%.

The secretion of PTH was measured in perifused parathyroid cells as described [5].

3. RESULTS

3.1. *Cytosolic calcium*

Resting $[Ca]_i$ in parathyroid cells at respectively 0.5 and 1.5 mM extracellular calcium was 258 ± 11 nM (SE) (n = 14) and 639 ± 36 nM (n = 20) ($P < 0.001$). At 1.5 mM calcium, 100 nM TPA and 100 nM 4β-PDD lowered $[Ca]_i$ to 335 ± 21 (n = 11) ($P < 0.001$) and 307 ± 17 nM (n = 4) ($P < 0.02$), respectively. A decrease of $[Ca]_i$ was seen within 1 min and it was complete by 4–5 min (fig.1). With 1 mM EGTA a similar decrease of $[Ca]_i$ was already seen within 2–3 min. The half-maximal dose of TPA lowering $[Ca]_i$ was 10 nM (not shown). A biologically inactive phorbol ester, 4α-PDD, lowered fluorescence slightly to an apparent $[Ca]_i$ of 492 ± 16 nM (n = 8), but the decrease was independent of the dose used (100 nM–5 μM) and not statistically significant ($P > 0.1$).

At 0.5 mM extracellular calcium TPA did not affect resting $[Ca]_i$. When the extracellular calcium concentration was increased in a stepwise manner from 0.5 to 3 mM in the absence and presence of 10 nM TPA, half-maximal elevations of $[Ca]_i$ were raised from 1.3 to 2 mM, and maximal $[Ca]_i$ reduced from 1000 to 500 nM, respectively (fig.2).

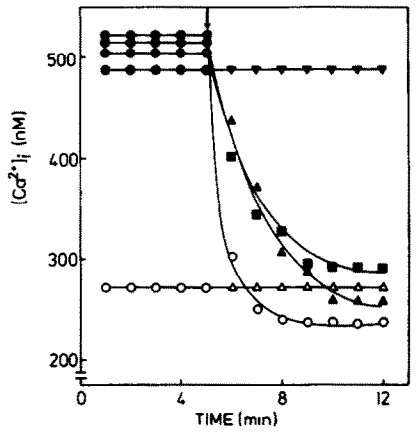

Fig.1. Effects of phorbol esters and EGTA in dispersed bovine parathyroid cells at 1.5 mM (●) and 0.5 mM (○) extracellular calcium on $[Ca]_i$. Arrows indicate addition of 1 mM EGTA (○), 100 nM TPA (▲, △), 100 nM 4β-PDD (■) and 100 nM 4α-PDD (▼). Traces are representative of 4–8 independent observations.

3.2. *Parathyroid hormone secretion*

Maximal stimulation of PTH secretion by 100 nM TPA at 1.5 mM extracellular calcium was the same as that obtained by lowering extracellular calcium from 1.5 to 0.5 mM (fig.3). As shown

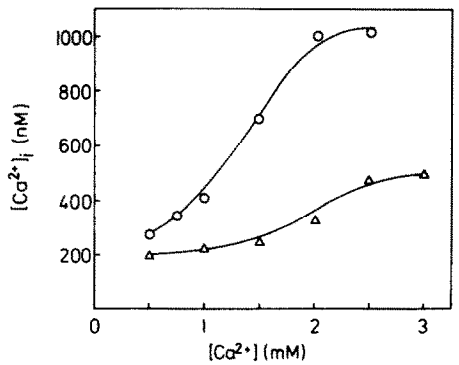

Fig.2. $[Ca]_i$ as function of medium calcium in the absence (○) and presence (△) of 10 nM TPA. Results are means of 4 (○) and 2 (△) independent observations.

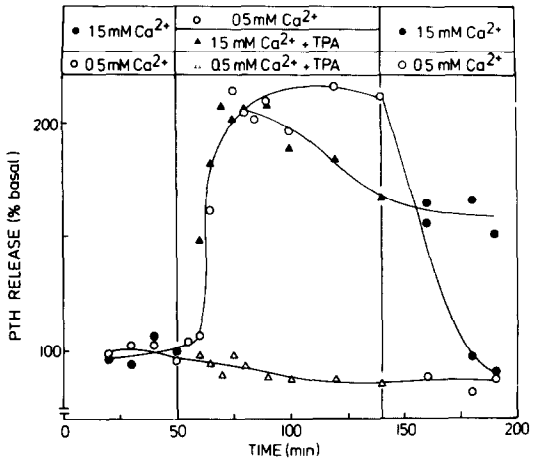

Fig.3. PTH stimulability of perifused parathyroid cells. Changes of media are indicated with different symbols. TPA was 100 nM. Results are representative of 6–10 observations.

earlier lowering of extracellular calcium caused a sustained increase of PTH secretion which was reversed by returning to 1.5 mM calcium [5]. In the presence of TPA, on the other hand, the release of PTH secretion was rapidly reduced to 60% of maximal levels and the raised secretion of PTH was unaffected by the removal of TPA for up to 50 min. At 0.5 mM calcium TPA was ineffective.

4. DISCUSSION

The inverse relationship between extracellular calcium concentrations and PTH release is unusual. Most endocrine cells require calcium for stimulation of hormone release, and stimulation by potassium and other agonists is in part mediated by $[Ca]_i$ (for references see [8]). However, evidence has also accumulated for exocytotic pathways not activated by raised $[Ca]_i$ [9].

In parathyroid cells the site of action of calcium and other divalent cations suppressing PTH secretion remains to be localized. Extracellular calcium and $[Ca]_i$ are proportionally related, which has led Brown et al. [4] to propose $[Ca]_i$ as a mediator of PTH secretory responses. Moreover, Brown et al. [2] have reported stimulation of PTH secretion by TPA in the absence of changes of cAMP production and $[Ca]_i$. In contrast to the latter findings, we have shown that TPA lowered $[Ca]_i$ and suppressed the rise of $[Ca]_i$ evoked by increased external calcium. The discrepant results cannot be readily explained. To this end, TPA has also been shown to lower $[Ca]_i$ in lymphocytes and cultured GH_3 pituitary cells [10,11], and inhibition of calcium uptake by TPA has been reported in HeLa cells [12]. Moreover, Lagast et al. [13] have observed ATP-dependent extrusion of calcium by TPA across the plasma membrane of neutrophils. The lowering of $[Ca]_i$ by TPA is not unique to parathyroid cells but may represent a negative feedback mechanism through which protein kinase C activity is regulated [14].

Release of PTH is maximally stimulated at 0.5 mM extracellular calcium [5,15]. At 0.5 mM calcium TPA did not raise PTH further suggesting that protein kinase C is maximally stimulated. Along these lines Morrissey [16] has observed suppression of diglyceride availability and in turn protein kinase C activity by high extracellular calcium concomitant with inhibition of PTH secretion. Furthermore, Brown et al. [2] also using dispersed bovine parathyroid cells and the present authors have observed the same PTH secretory response to TPA, but yet unaffected and lowered $[Ca]_i$, respectively. The results imply that the observed changes of $[Ca]_i$ are not responsible for the PTH secretory responses, but may be secondary to protein kinase C activation.

Thus, changes in $[Ca]_i$ may not mediate PTH secretory responses to changes of external calcium. A plasma membrane calcium sensor remains to be elucidated on parathyroid cells. Similar to calcium, La^{3+}, not permeating the plasma membrane, has been shown to inhibit diglyceride production and PTH secretion [17]. Moreover, Posilico et al. [18] have recently recognized modulation of PTH secretion and $[Ca]_i$ by anti-cell surface antibodies.

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